B4

CLAIMS

1. Heat-sensitive medium for the separation of species in a separating channel, the said medium comprising an electrolyte in which at least a set of block copolymers is dissolved, characterized in that the said block copolymers:

- are provided in the said electrolyte at a sufficient concentration to confer on the said medium the ability to reversibly transit from a viscosity state V1, obtained at a temperature T1, to a viscosity state V2 which is at least 100% higher than V1, obtained at a temperature T2 which is at least 20°C higher than T1 and

- comprising in their structure at least

  - two noncontiguous polymeric segments exhibiting an LCST in the said electrolyte and possessing an average number of atoms along their skeleton which is greater than 50, and

  - a polymeric segment which is soluble in the electrolyte at the temperatures T1 and T2.

2. Medium according to Claim 1, characterized in that the temperature T1 is between 15°C and 30°C.

3. Medium according to Claim 1 or 2, characterized in that the temperature T2 is between 40°C and 80°C.

4. Medium according to one of Claims 1 to 3, characterized in that the viscosity V2 is greater than the viscosity V1 by at least a factor equal to 5 at the viscosity V1.

5. Medium according to one of Claims 1 to 4, characterized in that the LCST of a significant fraction of the said segments with LCST is between T1 and T2.

6. Medium according to one of the preceding claims, characterized in that all the segments with LCST represent between 2% and 25% and preferably between 5 and 15% of the total average molar mass of the copolymers.

7. Medium according to one of the preceding claims, characterized in that all or some of the blocks with LCST possess along their skeleton an average number of atoms greater than 75, or an average molecular mass greater than 2 500.

8. Medium according to one of Claims 1 to 7, characterized in that all or some of the said polymers exist in the form of linear block polymers.

9. Medium according to one of Claims 1 to 7, characterized in that all or some of the said polymers exist in the form of comb copolymers whose skeleton consists of one or more segments which are soluble in the electrolyte at the temperatures T1 and T2.

10. Medium according to one of the preceding claims, characterized in that all or some of the copolymers possess an average number of segments with LCST per chain greater than 2 and preferably greater than 5.

11. Medium according to one of the preceding claims, characterized in that all or some of the copolymers possess a molecular mass greater than 30 000 or a number of atoms along the main skeleton greater than 2 000.

12. Medium according to one of the preceding claims, characterized in that all or some of the copolymers possess a molecular mass of between 50 000 and 3 000 000 or a number of atoms along the main skeleton of between 2 500 and 100 000.

13. Medium according to one of the preceding claims, characterized in that all or some of the copolymers possess an average number of atoms along a section of soluble segment, between two consecutive binding points of the said soluble segment with segments with LCST, greater than 210.

14. Medium according to one of the preceding claims, characterized in that all or some of the said polymeric segments with LCST are derived from one or more copolymers chosen from:

- polyvinyl alkyl ethers,
- hydroxyalkyl celluloses,
- homopolymers of ether oxides,
- random and block copolymers of ether oxides,
- alkylene homo- and copolymers, and
- polyacrylic derivatives derived from the homopolymerization or copolymerization of monomers chosen from acrylic and methacrylic acids, alkylacrylates and methacrylates, N-alkyl-acrylamides or -methacrylamides, N',N-dialkyl-acrylamides or -methacrylamides, aryl-acrylamides or -methacrylamides and alkylaryl-acrylamides or -methacrylamides.

15. Medium according to one of the preceding claims, characterized in that the polymeric segment(s) soluble at the temperatures T1 and T2 consist of at least one polymer chosen from polyethers, polyesters, soluble random copolymers and homopolymers of the polyoxyalkylene, polysaccharides, polyvinyl alcohol, polyvinylpyrrolidone, polyurethanes, polyamides, polysulphonamides, polysulphoxides, polystyrenesulphonate, substituted or unsubstituted

polyacrylamides or polymethacrylamides which are soluble in the said electrolyte.

16. Medium according to one of the preceding claims, characterized in that the copolymer is chosen from:

- copolymers of the comb copolymer type whose skeleton is of the type including acrylamide, acrylic acid, acryloylaminoethanol or dimethacrylamide and on which there are grafted side segments of the poly(N-alkyl or N,N-dialkyl)acrylamide type, or side segments of the random or block, polyoxyethylene/oxypropylene copolymer or polyoxypropylene type, or side segments of the polyether type

- copolymers of the block copolymer type exhibiting along their skeleton an alternation of segments of the polyoxyethylene type and of segments of the polyoxypropylene type, or an alternation of segments of the polyoxyethylene type and of segments of the polyoxybutylene type or an alternation of segments of polyethylene and of segments of the polyether type which are more hydrophobic than polyoxyethylene.

17. Medium according to one of the preceding claims, characterized in that the copolymer is chosen from

polyacrylamide/poly(N-isopropylacrylamide) (PAM-NIPAM); polyvinylalcohol/poly(N-isopropylacrylamide) (PVA-NIPAM), polyoxyethylene/polyoxypropylene, polyacrylamide/oxyethylene-oxypropylene copolymer, polyacrylamide/polyoxypropylene, polyacrylic acid/polyoxypropylene, polyacrylic acid/oxyethylene-oxypropylene copolymer, polyacrylic acid/poly(N-isopropylacrylamide) and polydimethylacrylamide/poly(N-isopropylacrylamide) (PDMAM-NIPAM).

18. Medium according to one of the preceding claims, characterized in that it transits from a viscosity V1 of between 50 and 1 000 $mPa.m^{-1}.s^{-1}$ (SI unit) at a temperature T1 of between 15 and 30°C to a viscosity V2 which is greater than V1 by a factor of between 2 and 50 at a temperature T2 of the order of 40°C or higher and in that it comprises between 5 g/100 ml and 20 g/100 ml of copolymers possessing

- an average molecular mass of between 30 000 and 2 000 000 or a number of atoms along the main skeleton of between 1 000 and 60 000,

- a fraction by mass of segments with LCST of between 2% and 20%, and

- an average molecular mass of the segments with LCST of between 2 000 and 20 000 or an avarage number of atoms along a segment with LCST of between 35 and 350.

19. Medium according to one of Claims 1 to 17, characterized in that it transits from a viscosity V1 of between 100 and 10 000 $mPa.m^{-1}.s^{-1}$ at a temperature T1 of between 15 and 30°C to a viscosity V2 which is greater than V1 by a factor of between 2 and 100 at a temperature T2 of the order of 40°C or higher and in that it comprises between 1 g/100 ml and 8 g/100 ml of copolymers possessing

- an average molecular mass of between 500 000 and 3 000 000 or a number of atoms along the main skeleton of between 7 000 and 90 000,

- a fraction by mass of segments with LCST of between 2.5% and 15%, and

- an average molecular mass of segments with LCST of between 4 000 and 30 000 or an average number of

atoms along a segment with LCST of between 60 and 600.

20. Medium according to one of Claims 1 to 17, characterized in that it transits from a viscosity V1 of between 100 and 10 000 $mPa.m^{-1}.s^{-1}$ (SI unit) at a temperature T1 of between 15 and 30°C to a viscosity V2 which is greater than V1 by a factor of between 2 and 100 at a temperature T2 of the order of 40°C or higher and in that it comprises between 0.1 g/100 ml and 5 g/100 ml of copolymers possessing

- an average molecular mass greater than 500 000 or a number of atoms along the main skeleton greater than 7 000,
- a fraction by mass of segments with LCST of between 2% and 15%, and
- an average molecular mass of the segments with LCST greater than 4 000 or an average number of atoms along a segment with LCST greater than 90.

21. Medium according to one of the preceding claims, characterized in that the said copolymer is present in the said medium and the copolymer concentration is less than 20 g/100 ml, and preferably between 0.1 g/100 ml and 8 g/100 ml.

22. Medium according to one of the preceding claims, characterized in that it comprises, in addition, adjuvants of the type including particles, water-soluble polymers, nonthermothickening associative polymers, or surfactants, which may be neutral or ionic.

23. Use of a medium according to one of the preceding claims, for the separation or analysis of species chosen from molecular or macromolecular species, and in

particular biological macromolecules such as nucleic acids (DNA, RNA, oligonucleotides), nucleic acid analogues obtained by chemical synthesis or modification, proteins, polypeptides, glycopeptides and polysaccharides, organic molecules, synthetic macromolecules or particles such as mineral particles, latex, cells or organelles.

24. Use of a medium according to one of Claims 1 to 22, for the sequencing of DNA.

25. Use according to Claim 24, characterized in that to separate molecules having a molecular mass of less than 50 000 or oligonucleotides comprising less than 100 nucleotides, or else native or denatured proteins, a medium according to Claim 18 is used.

26. Use according to Claim 24 or 25, characterized in that to separate products of reaction of DNA sequences, DNA duplexes of less than 1 000 base pairs, denatured proteins or synthetic or natural polymers having a molecular mass of between 20 000 and 1 000 000, a medium according Claim 19 is used.

27. Use according to Claim 24, characterized in that to separate DNA duplexes having a size of between 500 bases and several millions of base pairs, or particles such as latexes, whole cells, whole chromosomes or organelles, a medium according to Claim 20 is used.

28. Use according to one of Claims 24 to 27, characterized in that it comprises the following steps:

- selecting the said separation medium according to the characteristics of the species to be separated;

- introducing this medium into a separating channel of an electrophoresis apparatus in a sufficient

quantity to constitute its separation medium, the said separating channel being maintained at a temperature in the region of the temperature T1;

- placing a significant proportion of the channel at the temperature T2, either prior to or following the introduction of a sample;

- introducing a quantity of sample at the inlet of the separating channel;

- carrying out the separation at a temperature of the order of T2 in the thermostated portion of the channel; and

- detecting the migration of the analytes initially contained in the sample.

29. Use of a medium according to one of Claims 1 to 23 in an automated electrophoresis apparatus.

30. Use of a medium according to one of Claims 1 to 23 in a microfluidic system.

31. Capillary electrophoresis device comprising, as separation medium, a medium according to one of Claims 1 to 23.

Add A2